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TECHNICAL MANUSCRIPT 239

INFECTION OF CONTROL MONKEYS WITH COCCIDIOIDES IMMITIS BY CAGING WITH INOCULATED MONKEYS

Richard H. Kruse
Theron D. Green
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UNITED STATES ARMY
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TECHNICAL MANUSCRIPT 239

INFECTION OF CONTROL MONKEYS WITH *COCCIDIOIDES IMMITIS* BY CAGING WITH INOCULATED MONKEYS

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Industrial Health and Safety Division
DIRECTORATE OF INDUSTRIAL HEALTH AND SAFETY

Project 1C622401A072

August 1965

In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

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ABSTRACT

Monkeys were inoculated with Coccidioides immitis and communally caged with control animals. Six experiments were performed to ascertain the transmissibility of the fungus. Cross-contamination occurs when incomplete air-washing does not eliminate a secondary fungal aerosol. However, a new air-washing technique eliminated the secondary aerosol. When this cross-contamination is eliminated there is no transmission of coccidioidomycosis.

I. INTRODUCTION

Impetus for evaluating animal-to-animal transmission of infectious microorganisms resulted when Wedum[1] tabulated cross-infections occurring in cagemate control animals. Survey of the literature revealed that there has been little systematic experimental work published evaluating animal-to-animal transmission of Coccidioides immitis. Some reports are conflicting. Jacobson[2] placed C. immitis-infected guinea pigs in a cage housing control guinea pigs, and fed a second group of control guinea pigs remnants of food from the infected guinea pig cage. After three months there was no cagemate or fomite transmission. Rosenthal and Elmore[3] reported cross-infection when guinea pigs were caged with guinea pigs infected by intratracheal instillation of spherules in sputum. Smith, Pappagianis, and Saito[4] stated that Rosenthal and Elmore failed to demonstrate endosporulating spherules or positive cultures in the control guinea pigs. They could not demonstrate cross-infection in their own experiments when control guinea pigs were caged with guinea pigs that had draining testicular coccidioidal infections. Friedman, Smith, and Berman[5] stated that there is deficient documentation for contagion with C. immitis from the report of Rosenthal and Elmore. Posadas[6] reported that monkeys will develop coccidioidomycosis when injected subcutaneously. In experiments conducted at Fort Detrick no infection occurred in control animals housed with monkeys and dogs infected by respiratory exposure of C. immitis.[7-10] However, Castleberry, Converse, and Del Favero[11] reported animal-to-animal transmission of C. immitis when an infant rhesus monkey developed coccidioidomycosis. The infant's mother had an ulcerated coccidioidomycotic lesion in the medial surface of the right forearm. After two months of intimate contact the infant developed pulmonary coccidioidomycosis.

This study was undertaken to furnish data on the hazards associated with monkeys exposed by different routes. Once this information is obtained, measures can be devised to reduce or eliminate these hazards.

II. MATERIALS AND METHODS

Monkeys were infected by arthrospores of C. immitis. Control monkeys were caged with the infected monkeys. In some experiments the air from a cage housing an infected monkey and a cagemate control was passed into a second cage housing a control monkey.

A. CULTURE

C. immitis strain Silveira was obtained from desiccated Sabouraud's agar cultures by the method described by Sinski et al.[12] Purity, viability, and concentrations of the harvested spores were ascertained by serial ten-fold dilutions of culture in 0.85% NaCl containing 0.01% triethanolamineoleate.

B. EXPERIMENTAL ANIMALS

Ninety-three monkeys (Macaca mulatta), of both sexes, weighing 2 to 4 kg, were used. Thirty-nine were infected by respiratory (whole-body) aerosol exposure, or by intravenous, subcutaneous, intraperitoneal, or intramuscular injection of arthrospores of C. immitis. Fifty-four were controls.

Monkeys were tranquilized with intramuscular injections (0.1 mg per kg body weight) of Serynl* as a convenient adjunct to safe handling of the animals.

C. INFECTING DOSE

Respiratory exposure was accomplished in five experiments by placing monkeys in an aerosol chamber within the gastight, ventilate cabinet[13] and generating an aerosol of dry arthrospores by compressed air. Exposure time was regulated so each monkey would inhale 500 arthrospores.[14] The entire body of each monkey was exposed to the aerosol. After this exposure, the animals were air-washed (methods to be described later) to reduce contamination of fur, and transferred to a ventilated animal cage.[15]

In a sixth experiment monkeys were injected with either 25 or 100 arthrospores suspended in 0.85% NaCl solution. The injection site was disinfected with 2% peracetic acid, and the inoculated animal was transferred from the cabinet to an open wire cage containing an uninoculated cagemate control.

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* Parke-Davis Co., Detroit, Michigan.

All procedures connected with the aerosol exposure, inoculations, and transfer to cages were done in a closed system of cabinets with attached gloves (Fig. 1) that protected the experimenters. Cage handlers and animal caretakers were protected by wearing ventilated head hoods.[1]

D. LABORATORY EXAMINATIONS

The monkeys were observed daily at the time of feeding. The following procedures were done at 2-week intervals: (i) coccidioidin sensitivity was determined by injecting 0.1 ml of undiluted coccidioidin* intradermally in an eyelid, (ii) a blood sample was withdrawn from the saphenous vein for detection of precipitins and complement-fixation antibodies,** and (iii) frontal X-rays were taken.*** Complete necropsies were performed on animals that died during the course of the experiment, and upon survivors sacrificed at the conclusion of the experiment. Samples of tissue were removed aseptically from the apical and diaphragmatic lobes of the lung, the spleen, the liver, and the heart, and examined microscopically for spherules. Sections of the tissues were triturated in 5 ml broth containing 1% Phytone (BBL) and 1% dextrose, and suspensions were plated on Mycophil agar (BBL) containing 0.5 mg cycloheximide, 100 units penicillin, and 125 µg streptomycin per ml, and incubated at 30 C. All plates were kept 25 days before being discarded as negative for C. immitis. Tissues were fixed in 10% formalin, impregnated in paraffin, sectioned, and stained with Giemsa, Gomori methenamide silver, and periodic-acid Schiff stains for histopathological examination.****

Thirty-six fecal specimens were obtained from 18 monkeys from whom C. immitis later was recovered at necropsy. These specimens were examined by preparing a fecal suspension in 0.85% NaCl and plating in triplicate on Mycophil agar containing antibiotics. No C. immitis was recovered.

E. AIR SAMPLING

The aerosol-challenged monkey and its normal control were placed together in a closed ventilated cage from which a 3-ft-long air duct led to a second cage containing another normal monkey. The air from the second cage went through a duct to a collecting exhaust manifold. One air-sampling port was located in the duct connecting the first and second cages, and another in the duct that exhausted air from the second cage.

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* From Dr. Charles E. Smith, School of Public Health, University of California, Berkeley, California.

** Tests performed by Major Robert L. Taylor, Walter Reed Army Institute of Research, Washington, D.C.

*** Taken by SP-5 Arthur L. Self, and interpreted by Lt. Col. Nelson R. Blemly, U.S. Army Medical Unit, Fort Detrick, Maryland.

**** Performed by Captains George A. Deauville and Michael J. Doherty, Pathology Division, Fort Detrick, Maryland.

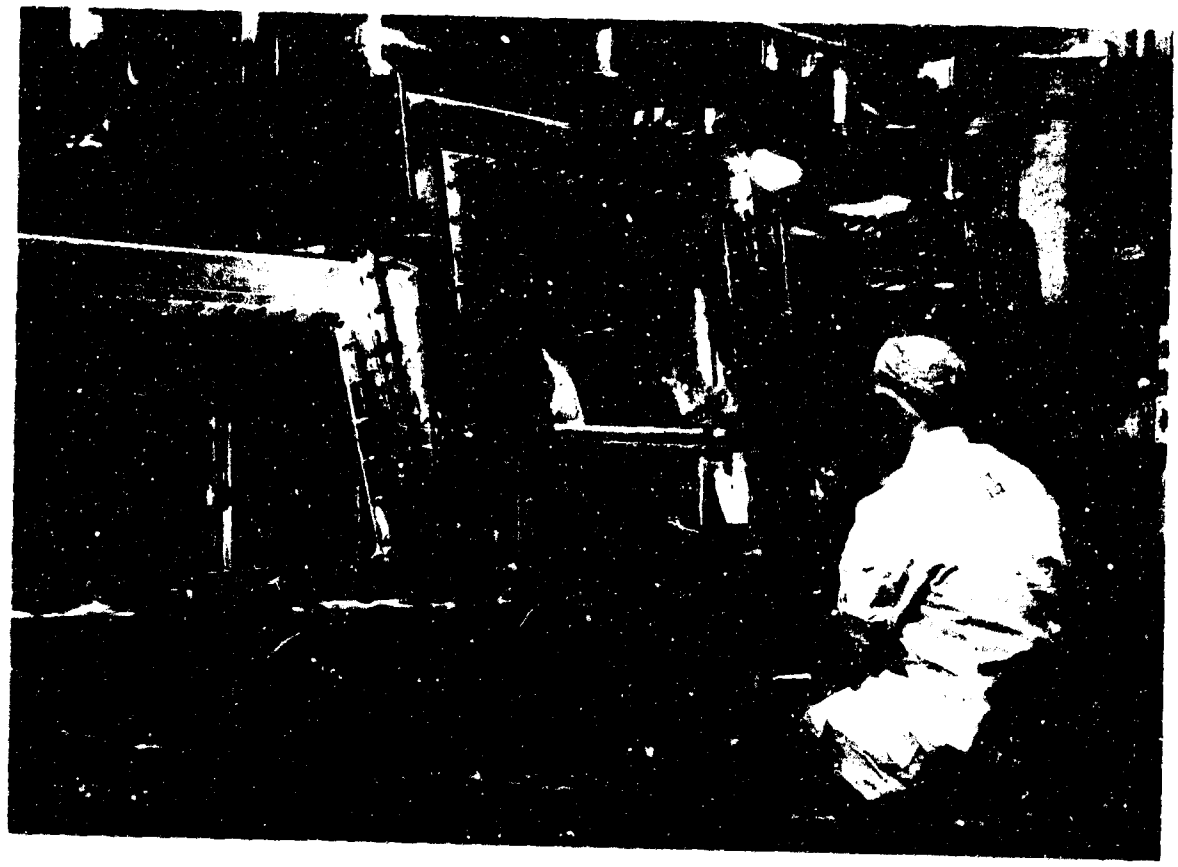

Figure 1. Exposing Monkey to Microbial Aerosol.

During the first 5 to 8 days after inoculation, at intervals of 4, 6, or 8 hr, 10 ft^3 of air was sampled from each port by a funneled sieve sampler[18] that contained an antibiotic Mycophil agar petri plate. All plates were incubated in the cabinet system at 30 C for 21 days and observed for development of colonies of C. immitis.

III. RESULTS

A. FIRST EXPERIMENT

Each of six monkeys separately inhaled a calculated dose of 500 dry arthrospores. Then each animal in turn was placed in the transfer cabinet (Fig. 2) where air intake and exhaust were regulated so that each animal was air-washed with 150 liters of air per min for 15 min. Each monkey next was moved into an attached, closed ventilated cage that housed an unexposed monkey. The transfer cabinet was disinfected with 2% peracetic acid. Then the cage housing the two animals was removed from the transfer cabinet, transported to the animal room, and connected by an air duct to another cage that housed another unexposed monkey (Fig. 3). Eighteen monkeys were caged in this way. A 50 FG deep-bed filter[17] in the first cage filtered the air from the animal room as it entered the cage. The cages were connected by air ducts so that air flow was from the room, through the 50 FG filter, through the cage housing the exposed monkey and its cagemate control, into the adjacent cage housing a separate control monkey, into the manifold, and through an absolute filter to the exhaust plenum. Airflow through the cages was maintained at 65 liters per min during the entire holding period.

Before another monkey was placed in the transfer cabinet the residual peracetic acid in the cabinet was neutralized by a spray of 0.5% $NaS_2O_3 \cdot 5H_2O$. After 5 min contact the cabinet was washed with water.

Air samples collected from exhaust ducts of the first and second cages housing the 18 monkeys showed that C. immitis was recoverable from the exhaust air duct of the first cage for as long as 108 hr after the aerosol-exposed monkey was placed in the cage, and from the air exhaust of the second cage for as long as 92 hr. The animals were sacrificed 40 days after aerosol challenge. All 18 monkeys, except one control (6C) in a second cage, were infected. Table 1 summarizes the results of the necropsies and laboratory examinations.

Figure 2. Air Washing Monke, in Transfer Cabinet.

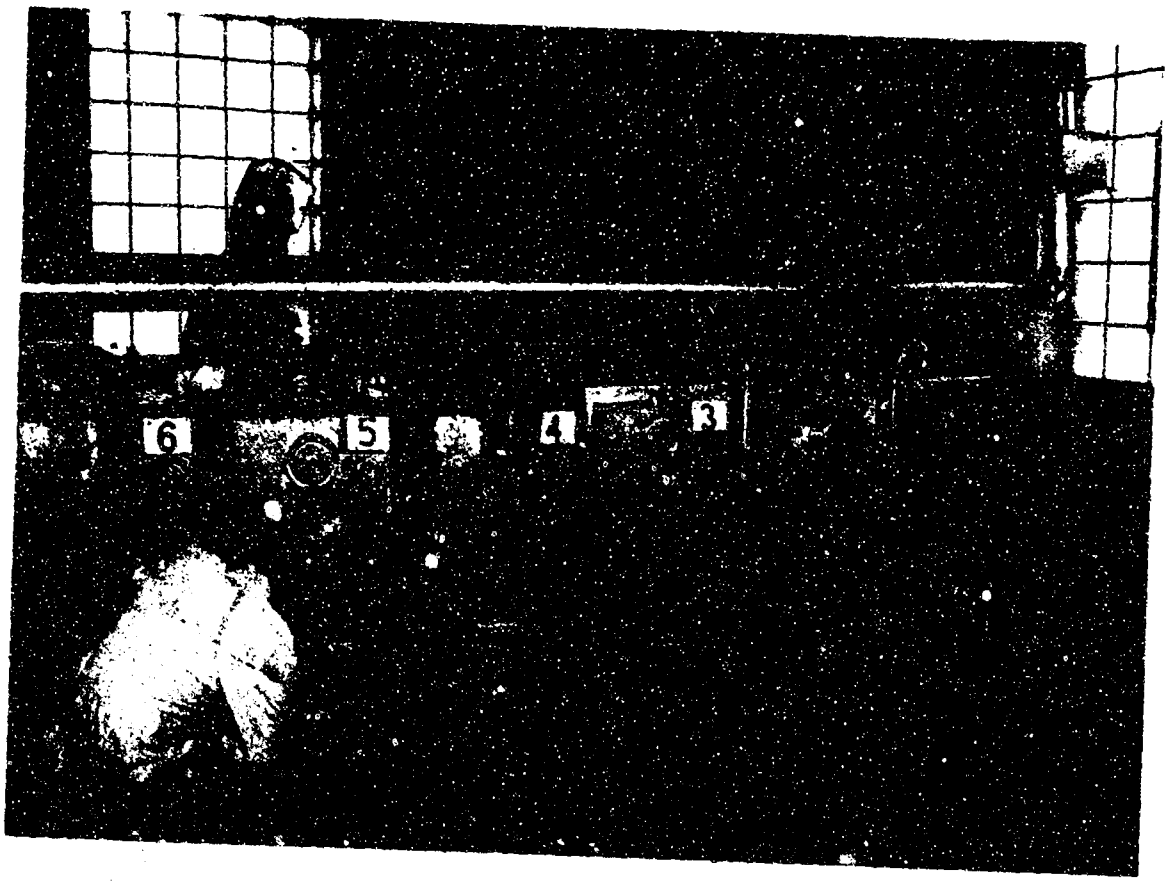

Figure 3. Ventilated Cages for Aerosol-Exposed Monkeys.

TABLE 1. MACACA MULATTA INHALING 500 DRY ARTHROSPORES OF COCCIDIOIDES IMMITIS AND AIR-WASHED 15 MINUTES

Monkey Number[a]	Coccidioidin Conversion	X-Ray Results	Highest		Necropsy		Histopathological Examination (Granulomatous Lesions with Spherules)
			Precipitin	CF[b] Titer	Spherules Present	C. immitis Cultured	
1A	+	+	1:20	1:16	-	+	-
1B	+	+	1:10	-	-	+	+
1C	+	+	-	-	+	+	+
2A	+	+	1:40	1:16	+	+	+
2B	+	+	1:40	-	-	+	+
2C	-	+	1:40	-	+	+	+
3A	+	+	1:20	-	-	+	-
3B	+	+	1:20	-	+	+	+
3C	-	+	1:10	-	+	+	+
4A	+	+	1:40	1:32	-	+	-
4B	-	+	-	-	+	+	+
4C	-	+	1:10	-	-	+	+
5A	+	+	1:40	1:8	+	+	+
5B	-	+	1:20	-	+	+	+
5C	-	+	1:20	-	-	+	+
6A	+	+	1:40	-	+	+	+
6B	-	+	1:40	-	+	+	+
6C	-	-	-	-	-	-	-

a. A = Exposed monkey in first cage.
B = Cagemate control of A.
C = Control in second cage.

b. Complement fixation.

B. SECOND EXPERIMENT (TABLE 2)

To determine if wiping the animal would reduce the secondary aerosol, three aerosol-challenged monkeys and six controls underwent the same procedure as in the first experiment except that air-washing was reduced to 10 min and the animals were wiped with a towel moistened with 2% quaternary ammonium compound.

C. immitis was recovered from the exhaust air of the first cage for 84 hr, and from the air of the second cage for 78 hr. When the nine monkeys were sacrificed 40 days after the aerosol challenge, all had contracted coccidioidomycosis.

TABLE 2. MACACA MULATTA INHALING 500 DRY ARTHROSPORES OF COCCIDIOIDES IMMITIS, AIR-WASHED 10 MINUTES, AND WIPED WITH 2% QUATERNARY AMMONIUM COMPOUND

Monkey Number[a]	Coccidioidin Conversion	X-Ray Results	Highest		Necropsy		Histopathological Examination (Granulomatous Lesions with Spherules)
			Precipitin	CF[b] Titer	Spherules Present	C. immitis Cultured	
1A	+	+	1:40	1:512	+	+	+
1B	-	-	-	-	-	-	-
1C	-	-	-	-	-	-	-
2A	+	+	1:40	1:512	-	+	+
2B	-	-	-	-	-	-	-
2C	-	-	-	-	-	-	-
3A	+	+	1:5	1:32	+	+	+
3B	-	-	-	-	-	-	-
3C	-	-	-	-	-	-	-

a. A = Exposed monkey in first cage.
B = Cagemate control of A.
C = Control in second cage.
b. Complement fixation.

C. THIRD EXPERIMENT (TABLE 3)

Would lengthening the air-wash reduce the secondary aerosol? Three aerosol-challenged monkeys and six controls were treated as in the first experiment except that this time, the three challenged animals were air-washed with 150 liters of air per min for 25 min.

C. immitis was recovered for 64 hr from the exhaust air of the first cage, and for 48 hr from the second cage. When the nine monkeys were sacrificed 60 days after aerosol challenge, all were infected.

TABLE 3. MACACA MULATTA INHALING 500 DRY ARTHROSPORES OF COCCIDIOIDES IMMITIS AND AIR-WASHED 25 MINUTES

Monkey Number[a]	Coccidioidin Conversion	X-Ray Results	Highest		Necropsy		Histopathological Examination (Granulomatous Lesions with Spherules)
			Precipitin	CF[b] Titer	Spherules Present	C. immitis Cultured	
1A	+	+	1:10	1:8	+	+	+
1B	-	+	-	-	-	+	+
1C	-	+	-	-	+	+	+
2A	+	+	1:40	1:16	+	+	+
2B	+	+	1:40	1:8	+	+	+
2C	-	+	1:20	-	+	+	+
3A	+	+	1:20	-	+	+	+
3B	+	+	-	-	-	+	+
3C	+	+	1:10	-	+	+	+

a. A = Exposed monkey in first cage.
B = Cagemate control of A.
C = Control in second cage.
b. Complement fixation.

D. FOURTH EXPERIMENT (TABLE 4)

The object of this experiment was to determine when the air in a cage housing an aerosol-exposed monkey would become noninfectious for a normal monkey, and therefore presumably also noninfectious for man. Each of 12 monkeys inhaled 500 arthrospores as before and was air-washed with 150 liters of air per min for 25 min. However, the 12 cagemate controls were not placed in the cages immediately. Twenty-four hr after the aerosol challenge, one non-exposed control monkey was placed in each of three cages housing an exposed monkey. At 48, 72, and 96 hr after exposure the other nine nonexposed monkeys were placed with the remaining nine exposed monkeys. The animals were sacrificed 60 days after aerosol exposure. C. immitis could not be recovered by air sampling the exhaust air after 64 hr. Control monkeys did not contract coccidioidomycosis when placed in the cages 72 and 96 hr after aerosol exposure.

E. FIFTH EXPERIMENT (TABLE 5)

A new method of air-washing was initiated. Again three monkeys were aerosol-challenged. The monkey was placed in the transfer cabinet and a flexible nozzle was adapted to the air line to replace the usual air flow of 150 liters per min through the cabinet. The air flow was then directed through the nozzle at the monkey to ruffle the fur. The animal was manipulated so that all parts of the body were air-washed by this forceful jet of air. After 10 min the nozzle was removed and the usual cabinet air was continued for five more min. These three monkeys and six controls were caged as in experiments 1, 2, and 3. The monkeys were sacrificed 60 days after aerosol exposure.

Sampling of the exhaust air recovered C. immitis up to 24 hr from cage 1, and up to 16 hr from cage 2. It should be noted that only one colony was recovered on one plate from cage 1 at 24 hr, and on one plate from cage 2. None of the control monkeys was infected.

TABLE 4. MACACA MULATTA INHALING 500 DRY ARTHROSPORES OF COCCIDIOIDES IMMITIS, AIR-WASHED 25 MINUTES, AND CAGEMATES PUT IN CAGES AT 24-HOUR INTERVALS

Monkey Number[a]	Coccidioidin Conversion	X-Ray Results	Highest		Necropsy		Histopathological Examination (Granulomatous Lesions with Spherules)
			Precipitin	CF[b] Titer	Spherules Present	C. immitis Cultures	
1A	+	+	1:40	1:1024	+	+	+
1B	+	+	1:10	1:128	+	+	+
2A	+	+	1:40	1:1024	-	+	+
2B	+	+	1:10	1:32	+	-	+
3A	+	+	1:20	1:128	+	+	+
3B	+	+	-	1:32	+	-	+
4A	+	+	1:20	1:128	-	+	+
4C	+	+	1:10	1:128	+	+	+
5A	+	+	1:20	1:512	+	+	+
5C	+	-	-	-	-	-	+
6A	+	+	1:20	1:256	+	+	+
6C	+	+	-	-	-	-	+
7A	+	+	1:40	1:512	+	+	+
7D	-	-	-	-	-	-	-
8A	+	+	1:40	1:1024	+	+	+
8D	-	-	-	-	-	-	-
9A	+	+	1:10	1:128	+	-	+
9D	-	-	-	-	-	-	-
10A	+	+	1:5	1:64	+	+	+
10E	-	-	-	-	-	-	-
11A	+	+	1:10	1:128	-	+	+
11E	-	-	-	-	-	-	-
12A	+	+	-	1:64	+	+	+
12E	-	-	-	-	-	-	-

a. A = Exposed monkey.
B = Cagemate control put in cage after 24 hr.
C = Cagemate control put in cage after 48 hr.
D = Cagemate control put in cage after 72 hr.
E = Cagemate control put in cage after 96 hr.

b. Complement fixation.

TABLE 5. MACACA MULATTA INHALING 500 DRY ARTHROSPORES OF COCCIDIOIDES IMMITIS, AIR-WASHED 10 MINUTES "RUFFLING THE FUR" AND 5 MINUTES NORMAL AIR-WASH

Monkey Number[a]	Coccidioidin Conversion	X-Ray Results	Highest Precipitin	Highest CF[b] Titer	Necropsy Spherules Present	Necropsy C. immitis Cultured	Histopathological Examination (Granulomatous Lesions with Spherules)
1A	+	+	1:20	1:512	+	+	+
1B	+	+	1:10	1:256	+	+	+
1C	+	+	1:10	1:64	-	+	+
2A	+	+	1:40	1:512	+	+	+
2B	+	+	1:20	1:128	+	-	+
2C	+	+	1:20	1:256	-	+	+
3A	+	+	1:40	1:512	+	+	+
3B	+	+	1:40	1:512	-	+	+
3C	+	+	1:10	1:64	-	+	+

a. A = Exposed monkey in first cage.
B = Cagemate control of A.
C = Control in second cage.
b. Complement fixation.

F. SIXTH EXPERIMENT (TABLE 6)

It seemed desirable to test whether normal control monkeys would be infected if caged with monkeys inoculated intravenously, subcutaneously, intramuscularly, or intraperitoneally. For each of these routes of inoculation, one monkey received 25 arthrospores and two monkeys received 500 arthrospores. The injection site was disinfected before and after injection, and the needle of the hypodermic syringe was surrounded by a cotton pledget soaked with 2% peracetic acid. Each of these animals was air-washed in the transfer cabinet as in the first experiment, and then moved into an open wire cage with an uninoculated monkey (Fig. 4). C. immitis was not recovered by air samples. The surviving monkeys were sacrificed after 60 days of communal housing. None of the controls was infected.

TABLE 6. MACACA MULATTA INOCULATED WITH ARTHROSPORES OF COCCIDIOIDES IMMITIS

Number	Route and Injection	No. of Arthrospores Injected	Coccidioidin Conversion	X-Ray Results	Maximum Precipitin	Maximum CF Titer	Necropsy: Spherules Found	Necropsy: C. immitis Cultured	Histopathological Examination: Granulomatous Lesions with Spherules
1A[a/]	ip	25	-	+	-	1:16	+	+	+
1B		0	-	-	-	-	-	-	-
2A	ip	100	+	+	1:40	1:256	-	+	+
2B		0	-	-	-	-	-	-	-
3A	ip	100	+	+	1:20	1:256	+	+	+
3B		0	-	-	-	-	-	-	-
4A[b/]	sc	25	+	+	-	1:16	+	+	+
4B		0	-	-	-	-	-	-	-
5A[b/]	sc	100	+	+	1:10	1:256	+	+	+
5B		0	-	-	-	-	-	-	-
6A[b/]	sc	100	+	+	1:5	1:256	+	+	+
6B		0	-	-	-	-	-	-	-
7A[c/]	iv	25	+	+	1:40	1:32	+	+	+
7B		0	-	-	-	-	-	-	-
8A[c/]	iv	100	+	+	1:40	1:128	+	+	+
8B		0	-	-	-	-	-	-	-
9A[d/]	iv	100	+	+	1:40	1:64	+	+	+
9B		0	-	-	-	-	-	-	-
10A[c/]	im	25	-	+	-	1:16	-	+	+
10B		0	-	-	-	-	-	-	-
11A	im	100	+	+	-	1:128	-	-	+
11B		0	-	-	-	-	-	-	-
12A	im	100	+	+	1:10	1:256	+	+	+
12B		0	-	-	-	-	-	-	-

a. Dead at 33 days.
b. Lesion at injection site.
c. Dead at 36 days.
d. Dead at 31 days.

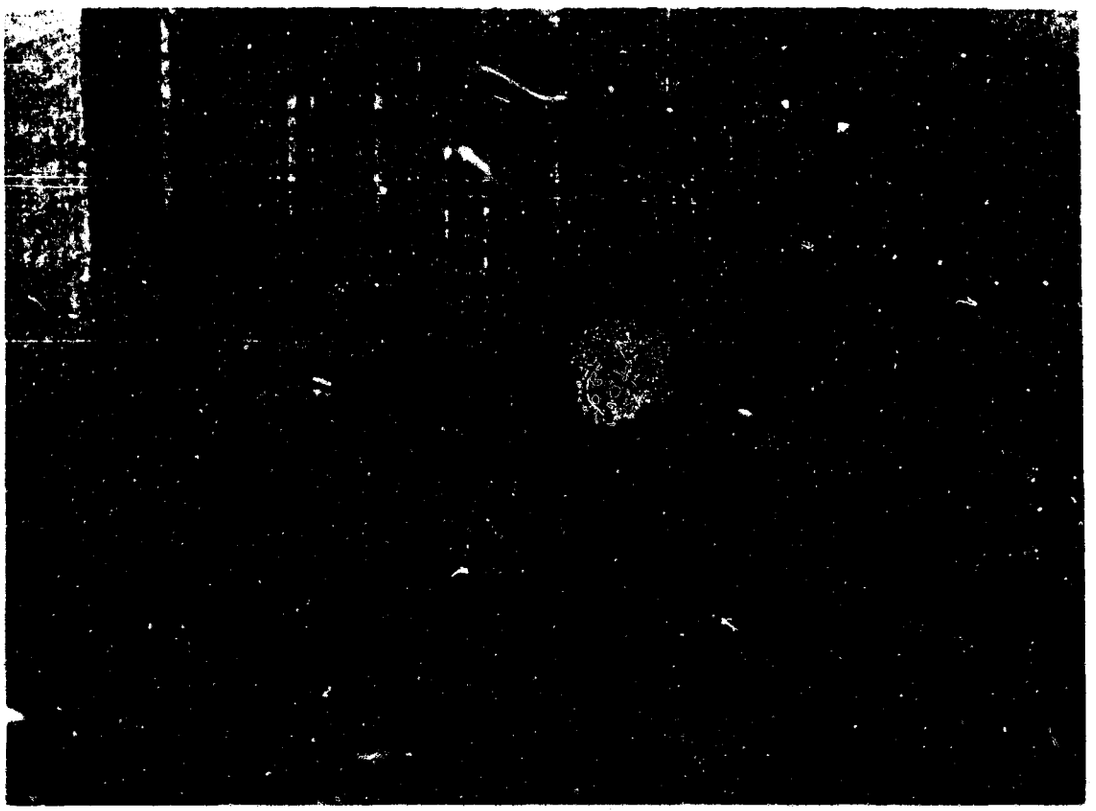

Figure 4. Cages for Parenterally Inoculated Monkeys.

IV. DISCUSSION

The method by which animals are infected by C. immitis in nature is not completely known. Maddy[18] concluded that spherules in feces, urine, saliva, and wound exudate were not directly infectious if inhaled, but that they were infectious after the spherules had ruptured and germinated infective arthrospores. Ahlfeldt[19] suggested the disease could be contracted by ingestion. Smith[20] and Lubarsky and Plunkett[21] found that ingestion of C. immitis did not cause infection. Blundell et al.[7] reviewed infections via the respiratory route, described the pathogenesis of coccidioidomycosis, and found that an inhaled dose of seven arthrospores would infect a monkey.

The term cross-infection can easily be misused. We believe a different term, cross-contamination, should be incorporated in transmission experiments, and that there should be a clear cut differentiation between cross-contamination and cross-infection. Cross-infection is the transmission of disease from an infected animal to a control animal. Cross-contamination is the transmission of microorganisms from an exposed animal to a control animal. This may occur by inhaling secondary aerosols, by physical contact between animals, by means of contaminated food or bedding, etc. In this study the waste-collecting pans in the cages were separated from the cage floor so that the monkeys could not reclaim dropped, and therefore possibly contaminated, food.[18]

Where does the secondary aerosol from an exposed animal originate? In these experiments, in which the entire body of the monkey was exposed to an infectious aerosol, the secondary aerosol came from the fur. This is proved by the difference between the results of experiments 1, 2, 3, and 4 and those in experiment 5.

The difference between cross-infection and cross-contamination is evident from data in Tables 1 through 5. In these experiments the exposed monkey was placed in the cage housing an unexposed control animal. The monkey did not have coccidioidomycosis, because it had been exposed only 15 or 25 min earlier, depending on the air-wash time. Normal air-washing did not eliminate arthrospores from the fur, as proved by sampling of the air from the cage air ducts and consequent recovery of C. immitis for as long as 108 hr after a 15-min air-wash, and for as long as 64 hr after a 25-min air-wash. Air sampling recovered C. immitis for 84 hr after the monkey had been given a 10-min air-washing and then wiped with a towel moistened with 2% QAC. No positive culture of C. immitis was obtained from swab samples of the monkeys and from the cage interior when taken at 2-week intervals. This finding coincides with the work of Sinski and Lowe* who took swab samples of monkeys in one of their experiments.

* Personal communication.

When the secondary aerosol is eliminated, cross-contamination does not occur and the control animals remain clinically uninfected (Tables 4 and 5). In experiment 4 the controls at 24-hr intervals were put into the cages housing an aerosol-exposed monkey. C. immitis was recovered for 64 hr from the air. During this time, controls B and C contracted coccidioidomycosis. But controls D and E, which were placed with exposed monkeys 72 and 96 hr postexposure, did not contract coccidioidomycosis because they were placed in their respective cages after the secondary aerosol had been eliminated. The exposed monkeys 7A through 12A now became clinically ill; nevertheless infection of their cagemates did not occur during the next 56 or 57 days (Table 4).

Furthermore, to prove that cross-infection and cross-contamination are not synonymous, parenterally injected animals were caged with normal monkeys (Table 6). By inoculating the animals in an area remote from the animal room, contamination of the fur was eliminated. Three monkeys inoculated subcutaneously developed draining lesions. C. immitis was cultured from the exudate. Although all the injected monkeys became infected, cross-infection of cagemate controls did not occur.

If air washing the exposed monkey would reduce secondary aerosol formation below the infective dose, cross-contamination would be eliminated. Forcibly ruffling the fur by air (Table 5) prevented cross-contamination, not only in cagemates, but in controls receiving air only from the cage housing the exposed animal and cagemate control. Simple air-washing is insufficient, because arthrospores remain fixed on the fur. Ruffling the fur will maintain safe caging and permit less cumbersome and expensive caging arrangements.

The data in this study show that there is no monkey-to-monkey transmission of coccidioidomycosis, regardless of whether the monkey is infected by aerosol challenge or by intravenous, subcutaneous, intraperitoneal, or intramuscular injection of arthrospores. However, cross-contamination does occur from whole-body, aerosol-exposed monkeys as a result of inhalation of a secondary aerosol that originates from the fur. The new air-washing procedure, consisting of a 10-min forceful air-ruffling of the fur, eliminates cross-contamination and prevents infection of cagemates. As a result, the danger of infecting an animal caretaker is greatly decreased, and the reliability of the experiment is increased.

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13 ABSTRACT

Monkeys were inoculated with Coccidioides immit. and communally caged with control animals. Six experiments were performed ascertain the transmissibility of the fungus. Cross-contamination occurs when incomplete air-washing does not eliminate a secondary fungal aerosol. However, a new air-washing technique eliminated the secondary aerosol. When this cross-contamination is eliminated there is no transmission of coccidioidomycosis.

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SECOND EXPERIMENT (TABLE 2)

To determine if wiping the animal would reduce the secondary aerosol, three aerosol-challenged monkeys and six controls underwent the same procedure as in the first experiment except that air-washing was reduced to 10 min and the animals were wiped with a towel moistened with 2% quaternary ammonium compound.

C. immitis was recovered from the exhaust air of the first cage for 84 hr, and from the air of the second cage for 78 hr. When the nine monkeys were sacrificed 40 days after the aerosol challenge, all had contracted coccidioidomycosis.

TABLE 2. MACACA MULATTA INHALING 500 DRY ARTHROSPORES OF COCCIDIOIDES IMMITIS, AIR-WASHED 10 MINUTES, AND WIPED WITH 2% QUATERNARY AMMONIUM COMPOUND

Monkey Number[a]	Coccidioidin Conversion	X-Ray Results	Highest		Necropsy		Histopathological Examination (Granulomatous Lesions with Spherules)
			Precipitin	CF[b] Titer	Spherules Present	C. immitis Cultured	
1A	+	+	1:10	1:8	+	+	+
1B	-	+	-	-	-	+	+
1C	-	+	-	-	+	+	+
2A	+	+	1:40	1:16	+	+	+
2B	+	+	1:40	1:8	+	+	+
2C		+	1:20	-	+	+	+
3A	+	+	1:20	-	+	+	+
3B	+	+	-	-	-	+	+
3C	+	+	1:10	-	+	+	+

a. A = Exposed monkey in first cage.
B = Cagemate control of A.
C = Control in second cage.
b. Complement fixation.

C. THIRD EXPERIMENT (TABLE 3)

Would lengthening the air-wash reduce the secondary aerosol? Three aerosol-challenged monkeys and six controls were treated as in the first experiment except that this time the three challenged animals were air-washed with 150 liters of air per min for 25 min.

C. immitis was recovered for 64 hr from the exhaust air of the first cage, and for 48 hr from the second cage. When the nine monkeys were sacrificed 60 days after aerosol challenge, all were infected.

TABLE 3. MACACA MULATTA INHALING 500 DRY ARTHROSPORES OF COCCIDIOIDES IMMITIS AND AIR-WASHED 25 MINUTES

Monkey Number[a]	Coccidioidin Conversion	X-Ray Results	Highest		Necropsy		Histopathological Examination (Granulomatous Lesions with Spherules)
			Precipitin	CF[b] Titer	Spherules Present	C. immitis Cultured	
1A	+	+	1:20	1:512	+	+	+
1B	+	+	1:10	1:256	+	+	+
1C	+	+	1:10	1:64	-	+	+
2A	+	+	1:40	1:512	+	+	+
2B	+	+	1:20	1:128	+	-	+
2C	+	+	1:20	1:256	-	+	+
3A	+	+	1:40	1:512	+	+	+
3B	+	+	1:40	1:512	-	+	+
3C	+	+	1:10	1:64	-	+	+

a. A = Exposed monkey in first cage.
B = Cagemate control of A.
C = Control in second cage.
b. Complement fixation.

TABLE 5. MACACA MULATTA INHALING 500 DRY ARTHROSPORES OF COCCIDIOIDES IMMITIS, AIR-WASHED 10 MINUTES "RUFFLING THE FUR" AND 5 MINUTES NORMAL AIR-WASH

Monkey Number[a]	Coccidioidin Conversion	X-Ray Results	Highest Precipitin	Highest CF[b] Titer	Necropsy Spherules Present	Necropsy C. immitis Cultured	Histopathological Examination (Granulomatous Lesions with Spherules)
1A	+	+	1:40	1:512	+	+	+
1B	-	-	-	-	-	-	-
1C	-	-	-	-	-	-	-
2A	+	+	1:40	1:512	-	+	+
2B	-	-	-	-	-	-	-
2C	-	-	-		-	-	-
3A	+	+	1:5	1:32	+	+	+
3B	-	-	-	-	-		-
3C	-	-	-	-	-	-	-

a. A = Exposed monkey in first cage.
B = Cagemate control of A.
C = Control in second cage.
b. Complement fixation.

F. SIXTH EXPERIMENT (TABLE 6)

It seemed desirable to test whether normal control monkeys would be infected if caged with monkeys inoculated intravenously, subcutaneously, intramuscularly, or intraperitoneally. For each of these routes of inoculation, one monkey received 2^5 arthrospores and two monkeys received 500 arthrospores. The injection site was disinfected before and after injection, and the needle of the hypodermic syringe was surrounded by a cotton pledget soaked with 2% peracetic acid. Each of these animals was air-washed in the transfer cabinet as in the first experiment, and then moved into an open wire cage with an uninoculated monkey (Fig. 4). C. immitis was not recovered by air samples. The surviving monkeys were sacrificed after 60 days of communal housing. None of the controls was infected.

TABLE 6. *MACACA MULATTA* INOCULATED WITH ARTHROSPORES OF *COCCIDIOIDES IMMITIS*

Monkey	Route and Injection	No. of Arthrospores Injected	Coccidioidin Conversion	X-Ray Results	Maximum Precipitin	Maximum CF Titer	Necropsy: Spherules Found	Necropsy: *C. immitis* Cultured	Histopathological Examination: Granulomatous Lesions with Spherules
1A[a]	ip	25	-	+	-	1:16	+	+	+
1B		0	-	-	-	-	-	-	-
2A	ip	100	+	+	1:40	1:256	-	+	+
2B		0	-	-	-	-	-	-	-
3A	ip	100	+	+	1:20	1:256	+	+	+
3B		0	-	-	-	-	-	-	-
4A[b]	sc	25	+	[illegible]	[illegible]	1:16	+	+	+
4B		0	-	[illegible]	-	-	-	-	-
5A[b]	sc	100	+	[illegible]	1:10	1:256	+	+	+
5B		0	-	-	-	-	-	-	-
6A[b]	sc	100	+	+	1:5	1:256	+	+	+
6B		0	-	-	-	-	-	-	-
7A[c]	iv	25	+	[illegible]	1:40	1:32	+	+	[illegible]
7B		0	-	-	-	-	-	-	[illegible]
8A[c]	iv	100	+	+	1:40	1:128	+	+	+
8B		0	-	[illegible]	-	-	-	-	-
9A[d]	iv	100	+	+	1:40	1:64	+	+	+
9B		0	-	-	-	-	-	-	-
10A[c]	im	25	-	+	-	1:16	-	+	+
10B		0	-	-	-	-	-	-	-
11A	im	100	+	+	-	1:128	[illegible]	-	+
11B		0	-	[illegible]	-	-	-	-	-
12A	im	100	+	+	1:10	1:256	+	+	+
12B		0	-	-	-	-	-	-	-

a. [illegible]ead at 33 days.
b. Lesion at injection site.
c. Dead at 30 days.
d. Dead at 31 days.